Ph.D. SYNOPSIS (2022)

RESEARCH STUDENT	**MR. RATHOD SANTOSH GINYANDEV**
MAIL ID.	santoshgrathod1506@gmail.com
MOBILE NO.	09545482880/09137688586
CATEGORY	V.J.N.T
SUBJECT	PHARMACEUTICAL SCIENCES & TECHNOLOGY
Ph.D. SYNOPSIS TITLE	IN SILICO AND IN-VIVO ASSESSMENT OF ANTI-DIABETIC ATTRIBUTES OF FRACTIONS AND PHYTOCHEMICALS OF FRUIT PEEL (S)
RESEARCH GUIDE	**Dr. DHAWALE SHASHIKANT C.**
MAIL ID.	shashiprathmesh@gmail.com
MOBILE NO.	09970700030 / 07020625083
DESIGNATION	PROFESSOR SCHOOL OF PHARMACY, S.R.T.M. UNIVERSITY, NANDED
RESEARCH CENTRE	SCHOOL OF PHARMACY, S.R.T.M.UNIVERSITY, NANDED
UNIVERSITY	SWAMI RAMANAND TEERTH MARATHWADA UNIVERSITY, NANDED-431606, (MAHARASHTRA STATE), INDIA
DATE OF R.A.C.	27/07/2021
DATE OF R.R.C.	05/04/2022

IN SILICO AND IN-VIVO ASSESSMENT OF ANTI-DIABETIC ATTRIBUTES OF FRACTIONS AND PHYTOCHEMICALS OF FRUIT PEEL (S)

A SYNOPSIS SUBMITTED FOR THE REGISTRATION OF DEGREE OF

DOCTOR OF PHILOSOPHY

In the subject of PHARMACEUTICAL SCIENCE
Submitted By

Mr. RATHOD SANTOSH GINYANDEV
M. Pharm.

Under the Guidance of

Dr. DHAWLE SHASHIKANT C.
M. Pharm., Ph.D.
Professor
SCHOOL OF PHARMACY,
S.R.T.M. UNIVERSITY, NANDED

स्वामी रामानंद तीर्थ
मराठवाडा विद्यापीठ,नांदेड.

Swami Ramanand Teerth Marathwada University,
Nanded-431606, (Maharashtra State), India
(2022)

INDEX

SR.NO.	CONTENT
01	ABSTRACT OF PROPOSED WORK PLAN
02	INTRODUCTION
03	REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT
04	OBJECTIVES OF RESEARCH
05	METHODOLOGY TO BE ADOPTED
06	IMPORTANCE OF STUDY
07	PROPOSED WORK PLAN
08	REFERENCES

ABSTRACT OF PROPOSED WORK PLAN

The present synopsis is for pharmacological evaluation of a selected plant part(s) extract for type-2 diabetes and its complications in experimental animals. After proper literature survey the selected plant part(s) will be collected at its flouring season. The selected plant part(s) will be authenticated, cleaned, dried and powdered for extraction with different solvents (Non-polar to Polar). Preliminary phytochemical investigation will be carried out; extract will be subjected for fractionation. The fractionated plant part(s) extract will then evaluated for acute toxicity study for the determination of safe dose (as per OECD guidelines) and screened for its preliminary hypoglycemic effect in animal models. The fractionated plant part(s) extract will then evaluated for antidiabetic activities in experimentally induced type-2 diabetes in animals. The obtained data will then statistically interpret, results will be analyzed and the research findings will be disseminated by publications for the health care providers and lay public.

INTRODUCTION

Diabetes and its complications are an increasingly important condition globally [1]. Where Asia in particular, is experiencing a rapidly emerging diabetes epidemic [2]. According to the World Health Organization (WHO) criteria, the prevalence of clinically diagnosed diabetes is reported to be 5.6% and 2.7% among urban and rural India, respectively [3]. A nationwide survey across India showed 1.3% prevalence of self-reported T2DM which is more in men (1.5%) than women (1.0%) [4]. A study in Western India indicated age standardized prevalence of 8.6% in urban [5] and 9.3% in rural Maharashtra [6]. According to a widely accepted estimation, the number of diabetic patients would reach 366 million by the year 2030 [7]. Diabetes mellitus is defined as a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both [8]. Type 1 diabetes mellitus is an autoimmune disease where immune system attacks and destroys the beta cells in the pancreas with little or no insulin production [9]. In type-2 diabetes (T2DM), also known as noninsulin-dependent diabetes mellitus, pancreas usually produces enough insulin but it cannot be effectively utilized by target organs, leading to a condition known as insulin resistance. Around 90–95% of the diabetic subjects are type-2 diabetic [10].

Currently, there are several oral hypoglycemic agents available for the treatment of diabetes mellitus recommended by American Diabetes Association (ADA) and European Diabetes Association for the Study of Diabetes (EASD). These drugs are administered orally and are thus also called oral hypoglycemic agents (OHAs) or oral antihyperglycemic agents. Among these, metformin is considered as first-line treatment [11] and sulfonylureas, thiazolidinedioners (TZDs), dipeptidyl peptidase-IV (DPP-4) inhibitors, glucagon-like polypeptide-1 (GLP-1) agonists and insulin are considered as second-line treatment [12]. These treatments are used either alone or in combination with other drugs to achieve better effects. Treatment is based on the interplay of patient's biochemical parameters and available therapeutic options [13]. With a wide range of newly available pharmacological agents, diabetes treatment has become more challenging and controversial owing to their undesirable side effects [11, 14].

In ancient literature, more than 800 plant species have been reported to have potent antidiabetic activity [15]. The three main domains of complementary and alternative medicine i.e. western herbalism, Chinese and Ayurvedic medicine are the main sources for the search of effective treatment of T2DM [16]. It has been reported that plants contain active compounds which act in synergistic fashion to produce the desired therapeutic effect [17]. The medicinal plants are also a rich source of antioxidants which are known to prevent/delay the development of diabetic complications [18]. Hence herbs are becoming convenient for diabetic treatment due to their efficiency, lesser side effects and relatively low costs as compared to allopathic drugs [19]. Herbs have several phytochemical constituents with different mechanisms of action like altered carbohydrate metabolism, regeneration of b-cells, insulin sensitizing effects, increasing glucose uptake and utilization etc. [20].

Medicinal plants are of great value in the field of medicine and cure of diseases. Practical experience and several modern research studies have shown that therapy using plants is better than using synthetic chemicals by being safer besides having synergistic effect of their active ingredients and presence of certain minerals and salts. There is still large number of medicinal plants in which all active constituents have not yet been investigated even though their medicinal effect is established by folklore and traditional system of medicine. The traditional system of medicine has utilized herbal formulations for treating various human ailments. The side effects of drugs of chemical origin have shifted focus of researchers towards natural therapeutics for curing health related problems in living beings. Although medicinal plant based herbal therapy is in existence from centuries but scientific validation is still a difficult challenge for developing herbal medicines. Multiple studies have shown that compounds and extracts derived from plants can cure or delay health related decline and extend lifespan and health span across a variety of species. Major hindrance in amalgamation of herbal medicine in modern medical practices is lack of scientific and clinical data proving their efficacy and safety. There is a need for conducting clinical research in herbal medicine, developing simple bioassays for biological standardization, pharmacological and toxicological evaluation and developing various animal models for toxicity and safety evaluation. It is also important to establish the active components from herbal extract.

- **Rational of the present Investigation**

The present investigation is an extension of the research work done at SCHOOL OF PHARMACY, S.R.T.M.UNIVERSITY, NANDED by Mr. Karle Pravin Prakash. Research work was entitled as "*BIOLOGICAL EVALUATION OF SELECTED PLANT PART(S) FOR TYPE-2 DIABETES AND ITS COMPLICATIONS IN EXPERIMENTAL ANIMALS*".

REVIEW OF LITERATURE AND DEVELOPMENT IN THE SUBJECT

➢ PREVIOUS WORK DONE IN THE RELEVANT AREA

Diabetes mellitus (DM) is a disease or chronic metabolic disorder with various etiologies; it is characterized by high plasma glucose levels with altered metabolism of carbohydrates, lipids and proteins as a result of insufficiency of insulin secretion and function (WHO, 1999). DM has been recognized as a medical exigency causing impaired micro and macro organ function. The changes depend on the severity and duration of hyperglycemia [21]. Uncontrolled DM is associated with complications like dyslipidemia, cardiomyopathy, nephropathy and neuropathy. Dyslipidemia represents a risk factor for cardiovascular disorders and contributes to the pathogenesis and complications in patients with DM [22]. Typically, the atherogenic profile in the diabetic population includes hyperglyceridemia, low levels of HDL and elevated LDL levels [23, 24]. During the progression of DM, decrease in weight of liver due to increased catabolic processes such as, glycogenolysis, lipolysis and proteolysis, which is the outcome of lack of insulin in the liver cells while the increase in weight of kidney has been reported due to glucose over-utilization and subsequent improvement in glycogen synthesis, lipogenesis and protein synthesis [25]. These changes could lead to serious micro-vascular nephritic complications, which involves a series of metabolic changes in the pathogenesis of diabetic nephropathy [26]. Over 40% of diabetic patients worldwide have been reported to develop severe diabetic nephropathy [27].

A current therapeutic approach using a synthetic analog such as insulin, oral hypoglycemic agents, i.e. bigunides, sulfonyl ureas, thiazolidinediones, alpha glucosidase inhibitors, DPP4- inhibitors etc. possesses the numerous side effect for the long-term control of DM. Due to the inability of the modern medicines to control the pathophysiological aspects of the DM, as well as the enormous cost it poses to the economy of the developing nations of the world, alternative strategies are urgently needed (WHO, 2002). The use of traditional medicinal plants could provide a good alternative in the management of DM and its complications, as it plays an important role in the lives of rural people, particularly in remote parts of developing countries which are poorly served with health facilities.

Indian plants which are most effective and the most commonly studied in relation to diabetes and their complications are; *Allium cepa, Allium sativum, Aloe vera, Cajanus cajan, Cocciniaindica,Caesalpiniabonducella,Ficusbengalenesis,Gymnemasylvestre,Momordicacharantia,Ocimumsanctum,Pterocarpusmarsupium,Swertiachirayita,Syzigiumcumini,Tinosporacordifolia and Trigonella foenum graecu*[28,29].In earlier research banana peel has been linked to antihyperglycemic properties. In our university research centre a potential antidiabetic activity of inner peel of various banana species have been found with significant antihyperglycemic and *in-vitro* antioxidant activity. In particular, ethanolic extracts of *Musa acuminata* reveled marked antihyperglycemic activity in Wistar rats. Similarly, *Musa acuminata* inner peels depicted noteworthy free radical scavenging activity [30].While, ethanolic extract of peels of *Musa cavendish* in alloxan-induced diabetic rats showed lipid lowering; nephroprotective and glucose lowering properties [31].Many plant constituents have varying degree of hypoglycemic and anti-hyperglycemic activity, among these are alkaloids, glycosides, galactomannan gun, polysaccharides, peptidoglycans, hypoglycans, guanidine, steroids, carbohydrates, glycopeptides, terpenoids, amino acids and inorganic ions[28].

- **LITERATURE REVIEW OF SOME SELECTED PLANTS**

Various parts of *Madhuca longifolia* including its leaves, bark have been shown to possess anti-hyperglycemic effects. Administration of 150 mg/kg and 300 mg/kg of hydroethanolic extract of the leaves of *Madhuca longifolia* (once a day, for thirty consecutive days) significantly lowered blood glucose levels in animals [32]. Furthermore, the activity of glucose-6-phosphate dehydrogenase, serum triglycerides, HDL and total cholesterol levels showed marked improvement indicating that the hydroethanolic extract of *Madhuca longifolia* leaves possesses antihyperglycemic activity [32]. An aqueous extract from *Madhuca longifolia* bark depicted its hypocholesterolaemic and hypotriglyceridaemic activities in Triton WR-1339 induced hyperlipemic rats as experimental model [29]. Administration of the methanolic extract of *Madhuca longifolia* bark in normal, glucose loaded and streptozotocin induced diabetic rats at a dose of 100 and 200 mg/kg body weight (p.o.) reveled a dose dependent hypoglycemic activity in all three animal models as compared with the standard antidiabetic agent glibenclamide at a dose of 500 µg/kg. The hypoglycemia produced by the *Madhuca longifolia* bark extract may be due to the increased glucose uptake at the tissue level and/or an increase in pancreatic β-cell function, or due to inhibition of intestinal glucose

absorption. Indicating methanolic extract of *Madhuca longifolia bark* to be a potential antidiabetic agent [33]. The ethanolic extract of the bark of *Madhuca longifolia* is a good source of compounds with antioxidant properties while the extract also exhibited significant free radical scavenging activity, reducing power activity and superoxide scavenging activity [33,34]. A number of triterpenoids including α and β amyrin acetates, 3 β monocaprylic ester of erythdiol, 3 β capryloxy oloanolic acid and acetate, have been isolated from the mesocarp of *Madhuca longifolia* fruit. The alcoholic extractive of the pulpy mesocarp of the fresh fruit on fractionation with hexane and subsequent column chromatography, yielded the hithero unreported 3 βmonocaprylic esters of erythrodiol and oleanolic acid, along with αand β amyrine acetates [35]. Major of the above phytochemicals have antidiabetic activity [36]. Studies have showed that the oral administration of α-amyrin acetate (3a) significantly improved the diabetic condition in streptozotocin-induced diabetic rats and in model type 2 diabetic mice at 50 mg/kg dose [37]. Several triterpenoid and steroidal glycosides have been isolated which stimulate insulin release and blocks the formation of glucose in the blood stream [38, 39]. The literature data reveled that *Madhuca longifolia* fruit have predominant active phytochemicals prone to possess hypoglycemic or antidiabetic activity, which could be pharmacologically evaluated.

Likewise, many literatures depicted *Manilkara zapota (L.)* plant parts such as fruit, leaves and seeds with hypoglycemic activity. The fruit reveled hypoglycemic effect through α-amylase (IC_{50} value = 53.0 µg/ml) and α-glucosidase (IC_{50} value = 56.0 µg/ml) inhibitory activities [40].The Proanthocyanidins {(epi)gallocatechins} in unripe *Manilkara zapota (L.)*were also shown to inhibit α-amylase with an IC_{50} value of 4.2 ± 0.2 µg/mL and α-glucosidase with an IC_{50} of 16.6 ± 0.3 µg/mL [41]. The alcoholic and aqueous extracts of the unripe fruits, in addition to their aqueous homogenate exhibited antioxidant, antihyperglycemic and hypocholesterolemic activities [42]. In a study significant hypoglycemic potency of the leave and seed extracts of *Manilkara zapota (L.)* was evidenced [43]. Previous research has indicated that unripe fruits of *Manilkara zapota (L.)* are an excellent source of antioxidants, with over 3000 mg of L-ascorbic acid equivalent antioxidant capacity (AEAC) per 100 g of fresh sample due to presence of 24 several antioxidants in the extract. The antioxidant capacity of *Manilkara zapota (L.)* fruits was mainly attributed to polyphenolics with basic blocks of gallocatechin or catechin or both [44]. *Manilkara zapota (L.)* fruit peel had

total polyphenol content (TPC) higher than the pulp and also rich in other bioactive compounds, which may act as effective antioxidants. Fruit peel had the lowest EC_{50} value as compared to its pulp, which corresponded to the highest level of scavenging effect, measured by DPPH assay [45]. Significant evidence suggests that antioxidant and polyphenol-rich diets have the ability to protect against diabetes. The dietary antioxidants and polyphenols are beneficial in type 2 diabetes as; they protect pancreatic β-cells against glucose toxicity, gives anti-inflammatory and antioxidant effects, inhibits α-amylases or α-glucosidases thus decreases starch digestion and inhibits advanced glycation end products formation [46].It is significant to improve the beneficial effects of dietary polyphenols and clinical outcomes for diabetics and to understand how to use dietary polyphenols for prevention of diseases related to type 2 diabetes. One of specific area of diabetes-polyphenol research is to evaluate novel phytoconstituent for improving the benefits of dietary polyphenols and clinical outcomes for type 2 diabetics. So the *Manilkara zapota (L.)* fruit peel extract could find a potent candidature to be pharmacologically evaluated for type 2 diabetics and its complications.

Green *Actinidia deliciosa* is one of the most popular fruits worldwide, and it has various biological properties, including antioxidant, antidiabetic, anti-allergic, and cardiovascular protective effects [47,48]. The methanol extract of green *Actinidia deliciosa* leaf revels the suppression of postprandial blood glucose level after an oral administration of soluble starch or sucrose in mice. The mechanism of action was proposed to be due to the α-amylase-inhibiting activity in the 90% aqueous methanol fraction, while α-glucosidase-inhibiting activity in the n-butanol fraction, proven in the results of in vitro experiments [49]. The methanolic extract of green *Actinidia deliciosa* fruit showed a remarkable decrease in blood glucose level in diabetic induced Albino Wister male rats [50]. The peel of *Actinidia deliciosa* fruit, which is a byproduct of processing, is a good source of flavonoids; however, its bioactivity has not been widely investigated. With antioxidant activity-guided fractionation of green *Actinidia deliciosa* fruit peel crude extracts, various individual constituents were isolated such as naringenin, quercetin, tricin, kaempferol, epicatechin, catechin, gallo-catechin, and rutin, along with vitamin E, 2,8-dimethyl-2-(4,8,12-trimethyltridec-11-enyl) chroman-6-ol, as well as α and δ-tocopherol, 7 sterols, the triterpene ursolic acid, chlorogenic acid, β-sitosterol [51,52,53]. So the literature data reveling that the green *Actinidia deliciosa* fruit peel have quercetin, epicatechin, catechin, gallo-catechin as predominant active phytochemicals prone to hypoglycemic or antidiabetic activity, with worth placing the green *Actinidia deliciosa* fruit peel as a potent candidate in research way of type-2 antidiabetics.

OBJECTIVES OF RESEARCH

Literature survey reveals that several plants have always been proven as a better source of drugs when it comes to treatment of diabetes and its complication. In literature study for this proposed synopsis it reveals that fruit/peel of some plants like *Manilkara zapota (L.)* with several 24 antioxidants and certain polyphenols, phytochemicals of green *Actinidia deliciosa* like quercetin, epicatechin, catechin, gallo-catechin along with other hypoglycemic phytochemicals and triterpenoids including α and β amyrin acetates in *Madhuca longifolia* are identified antiglycemic phytoconstituent which could pharmacologically be evaluated for type-2 diabetes. Hence, it was thought worth to undertake present study to screen anti-diabetic activities of one of the fruits.

The broader objectives set for the pre-clinical studies are as follows-

- To evaluate the selected fruit peel extract for effective control of type-II diabetes mellitus and its long term complications as a therapeutic/supplementary medicine.
- To extract the fruit peel using proper solvent(s).
- To isolate the phytochemicals by using scientific methods.
- To characterize the isolated phytochemicals with the help of different techniques.
- To perform the docking study for phytochemicals.
- To perform the in silico activity of phytochemicals.
- To evaluate the pharmacological activities of fruit peel (s) extract.
- To evaluate the effect of the anti-diabetic principle(s) of phytochemicals on glucose tolerance.
- To rationalize the ethanomedical information by statistically proving its effects pre-clinically.
- To disseminate of the research findings by publications for the health care providers and lay public.

It is expected that this study could provide a scientific basis for the use of a fruit peel extract in herbal medicine to ameliorate the complications of diabetes mellitus.

METHODOLOGY TO BE ADOPTED

1. ***Collection and authentication of selected fruit peel (s)***

   The plant material will be collected, authenticated, cleaned, dried and powdered for further process.

2. ***Preliminary Phytochemical Investigation***

   A. Extraction

   The fruit peel will be subjected for extraction with different polarity solvents (Non-polar to Polar)

   B. Preliminary phytochemical investigation

   Preliminary phytochemical investigation of fruit peel extract will have carried out by qualitative chemical test and chromatographic technique.

3. ***Acute toxicity study***

   For the determination of safe dose, the fruit peel extract will be subjected for the acute toxicity according to OECD guidelines.

4. ***Evaluations of hypoglycemic and antihyperglycemic potential of fruit peel extract***

   The fruit peel extract will be subjected for study of hypoglycemic and antihyperglycemic activity using preliminary screening in experimental animal.

5. ***Evaluations of anti-diabetes activity of fruit peel extract***

   The fruit peel extract will be subjected for study of anti-diabetes activity using various type-2 diabetes in animal models.

6. ***Evaluations of anti-cancer activity of fruit peel extract***

   The fruit peel extract will be subjected for study of anti-cancer activity using various *in-vitro* models.

IMPORTANCE OF STUDY

➢ INTERNATIONAL STATUS

Diabetes mellitus is a metabolic disorder widespread in all parts of the world and is becoming a serious threat for mankind. The global use of complementary and alternative medicine (CAM) for the management of diabetes has rapidly increased over the last decade. It is reported that up to 72.8% of people with diabetes uses herbal medicine, dietary supplements and other CAM therapies [54]. Furthermore, research indicates that most people who use CAM therapies do so in addition to, rather than in place of, conventional medicine [55]. A large number of medicinal plants are believed to possess antidiabetic properties and have been utilized to manage diabetes [56]. There are lots of chemical agents available for controlling & treating diabetic patients, but total recovery from diabetes has not been reported up to this date. In addition to adverse effects, drug treatments are not always satisfactory in maintaining euglycemia (normal concentration of glucose level in blood) and avoiding late stage diabetic complications. Alternative to these synthetic agents, plants provide a potential source of hypoglycemic drugs and are widely used in several traditional systems of medicine to treat this disease or are used as adjuvants to many allopathic antidiabetic drugs. Several medicinal plants have been investigated for their beneficial effects in different types of diabetes. Overall there has been great demand for plant products due to low cost, easy availability and lesser side effects. One of the etiologic factors implicated in the development of diabetes and its complications is the damage induced by free radicals and hence antidiabetic herbs with antioxidant properties are found be more beneficial worldwide [57].

➢ NATIONAL STATUS

Now a day in India alternative therapies with anti-hyperglycemic effects are urging for the management of T2DM. Although herbal medicines are commonly used as a complementary therapy, herb-drug interactions are being analyzed and evaluated by carrying out randomized controlled clinical trials. The preparation of standardized herbal medicines is urgently needed for future studies and therapies. Variety of herbal formulations are used as therapeutic agents to treat T2DM [58]. The most effectively used antidiabetic herbal formulations in Indian market are, Alangiumsalvifolium tablet, Bitter gourd tablets, D-400 tablet, Diabecon, Diabecure,Diabet capsule ,Dia-care ,Diamed powder ,Dianex powder ,Diashis powder, Diasulin,Diasulin powder ,Dihar powder ,Dihar powder ,Epinsulin,Gurmar powder ,Hyponidd powder , Ipomoea digitata tablet ,Madurishtchurna, MTEC powder , Pan five powder ,Syndrexetc. Combinational therapy with omega-3 fatty acid supplementation are find to be helpful in diabetes management, its complications while reducing polypharmacy [59].

➢ SIGNIFICANCE OF THE STUDY

Diabetes requires early diagnosis, treatment, and lifestyle changes. Diabetes is a disease that affects many people in the 21stcentury and is known as the fifth leading cause to death. High prevalence, variable pathogenesis, progressive process, and complications of diabetes all highlight the urgent need for effective treatments. Nowadays different treatments such as insulin therapy, pharmacotherapy, and diet therapy are available to control diabetes. There are several types of glucose-lowering drugs that exert anti-diabetic effects through different mechanisms. These mechanisms include stimulation of insulin secretion by sulfonylurea and meglitinides drugs, increasing of peripheral absorption of glucose by biguanides and thiazolidinediones, delay in the absorption of carbohydrates from the intestine by alpha-glucosidase, and reduction of hepatic gluconeogenesis by biguanides. In the past three decades, despite the significant progress made in the treatment of diabetes, the results of treatment in patients is still far from perfect. These treatments have some disadvantages, including drug resistance (reduction of efficiency), side effects, and even toxicity. For example, sulfonylureas lose their effectiveness after 6 years of treatment in 44% of patients. It is also said that the glucose-lowering drugs are not able to control hyperlipidemia [60].

Most tests have demonstrated the benefits of medicinal plants containing hypoglycemic properties in diabetes management. The most common herbal active ingredients used in treating diabetes are flavonoids, tannins, phenolic, and alkaloids [61,62]. The existence of these compounds implies the importance of the anti-diabetic properties of these plants [61]. For example, tannin improves the function of pancreatic β-cells and increases insulin secretion. Quercetin is an antioxidant that acts in several mechanisms related with the removal of oxygen radicals, so prevents lipid peroxidation and metal ion chelation [61,62]. In fact, the mechanisms of actions for hypoglycemic plants include: increasing of insulin secretion, increasing of glucoses absorption by muscle and fat tissues, prevention of glucose absorption from the intestine, and prevention of glucose production from liver cells [63]. These factors are mostly responsible for the reduction or elimination of diabetes complications. Despite the presence of anti-diabetic drugs in the pharmaceutical market, the treatment of diabetes with medicinal plants is often find successful. Herbal medicines and plant components with insignificant toxicity and no side effects are notable therapeutic options for the treatment of this disease around the world [61,62].

➢ NEED FOR THE STUDY

In modern days, huge attention has been directed towards recognition of plants with antidiabetic ability that may be used effectively for human consumption. There has been rapid development of different classes of antihyperglycemic drugs with distinctive pharmacological mechanism of action and also they have various toxicological profiles. Numbers of medications are reported for the management of hyperglycemia like insulin, sulphonylureas, biguanides, thiazolidinedione, alpha-glucosidase inhibitors, glucagon-like peptide-1 analogues, glycosourics and dipeptidyl peptidase- IV inhibitors. Side effect of antihyperglycemic drugs may cause agitation, altered behavior, excess sweating, inaudible speech, tachycardia, seizures, and coma. Side effect of subcutaneous dose of insulin is related with deep risk blurred vision and hypoglycemia. Sulfonylureas can cause hypoglycemia, which stimulates appetite and leads to weight gain. Biguanides causes anorexia and encourage weight loss. Thiazolidinediones like pioglitazone, rosiglitazone can cause hepatic dysfunctioning as an adverse effect on regular treatment. Still there is a challenge to the medical system for management of diabetes without any side effects. However, natural remedies are widely used around the world to treat diabetes. So, the search for natural drugs from medicinal plants is being increased because of its fewer side effects, willingly availability and low cost. Thus the scientific validation of medicinal plants traditionally used in the treatment and management of diabetes is demanded.

The present synopsis claims for pharmacological evaluation of *Manilkara zapota (L.)* fruit peel **or** green *Actinidia deliciosa* fruit (or peel) **or***Madhuca longifolia* fruit (or peel) in type 2 diabetics and its complications with animal models. The previous studies reveled several 24 antioxidants and certain polyphenols in *Manilkara zapota (L.)* fruit peel corresponding to the highest level of free radical scavenging effect. Green *Actinidia deliciosa* fruit (or peel) constitutes quercetin, epicatechin, catechin, gallo-catechin along with other hypoglycemic phytochemicals, while a number of triterpenoids including α and β amyrin acetates in *Madhuca longifolia* fruit (or peel). Thus these identified antiglycemic phytoconstituent in above mentioned plant part(s) needs to be pharmacologically evaluated in type-2 diabetic experimental animals.

PROPOSED WORK PLAN

Sr. No.	RESEARCH WORK		TENTATIVE TIME IN MONTH(S)
1.	Literature survey		03
2.	Selection, Collection and authentication of plant material(s)		02
3.	Extraction of fruit peel (s)		02
4.	Preliminary phytochemical evaluation of fruit peel (s) extract		02
5.	Pharmacological evaluations		
	1.	Acute Toxicity Study for the determination of safe dose (as per OECD guidelines)	01
	2.	Evaluation of antidiabetic activity	
	a.	Preliminary study 1) Antihyperglycemic study in non-diabetic rats 2) Hypoglycemic study in non-diabetic rats	04

	b.	Antidiabetic study 1) Chronic study in experimentally induced type-2 diabetic animal models.	06
		2) Study in experimentally induced insulin resistance in animal model(s).	06
		3) In vitro Glucose uptake in rat isolated hemi-diaphragm.	01
6.	Statistical interpretation and result analysis		02
7.	Research publication		06
8.	Completion of research work and thesis writing		03

REFERENCES

1. Whiting DR, Guariguata L, Weil C, Shaw J. IDF diabetes atlas: global estimates of the prevalence of diabetes for 2011 and 2030. Diabetes research and clinical practice. 2011 Dec. 31;94(3):311-321.
2. Yang W. Changing characteristics of the type 2 diabetes epidemic of China and other Asian countries. Journal of diabetes investigation. 2013 May 1;4(3):223-224.
3. Mohan V, Pradeepa R. Epidemiology of Diabetes in Different Regions of India. Health administrator. 2009;22(1-2):1-8.
4. Upadhyay RP, Misra P, Chellaiyan VG, Das TK, Adhikary M, Chinnakali P, Yadav K, Sinha S. Burden of diabetes mellitus and prediabetes in tribal population of India: a systematic review. Diabetes research and clinical practice. 2013 Oct. 31;102(1):1-7.
5. Gupta A, Gupta R, Sarna M, Rastogi S, Gupta VP, Kothari K. Prevalence of diabetes, impaired fasting glucose and insulin resistance syndrome in an urban Indian population. Diabetes research and clinical practice. 2003 Jul. 31;61(1):69-76.
6. Deo SS, Zantye A, Mokal R, Mithbawkar S, Rane S, Thakur K. To identify the risk factors for high prevalence of diabetes and impaired glucose tolerance in Indian rural population. International journal of diabetes in developing countries. 2006 Jan. 1;26(1).
7. Kaushik G, Satya S, Khandelwal RK, Naik SN. Commonly consumed Indian plant food materials in the management of diabetes mellitus. Diabetes & Metabolic

Syndrome: Clinical Research & Reviews. 2010 Mar. 31;4(1):21-40.

8. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2014 Jan. 1;37(1): S 81-90.
9. Jacobsen IB, Henriksen JE, Hother-Nielsen O, Vach W, Beck-Nielsen H. Evidence-based insulin treatment in type 1 diabetes mellitus. Diabetes research and clinical practice. 2009 Oct. 31;86(1):1-10.
10. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes care. 2014 Jan. 1;37(1): S81-90.
11. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, Peters AL, Tsapas A, Wender R, Matthews DR. Management of hyperglycemia in type 2 diabetes, 2015: a patient-centered approach: update to a position statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes care. 2015 Jan. 1;38(1):140-149.
12. Nicholson G, Hall GM. Diabetes mellitus: new drugs for a new epidemic. British journal of anesthesia. 2011 May 24;107(1):65-73.
13. Stein SA, Lamos EM, Davis SN. A review of the efficacy and safety of oral antidiabetic drugs. Expert opinion on drug safety. 2013 Mar. 1;12(2):153-175.
14. Ghadge AA, Harke SM, Khadke SP, Diwan AG, Pankaj M, Kulkarni OP, Ranjekar PK, Kuvalekar AA. Circulatory adipocytokines and lipid profile variations in type-2 diabetic subjects: desirable side-effects of antidiabetic drugs. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2014 Dec. 31;8(4):230-232.
15. Pandey A, Tripathi P, Pandey R, Srivatava R, Goswami S. Alternative therapies useful in the management of diabetes: A systematic review. Journal of pharmacy & bioallied sciences. 2011 Oct.;3(4):504.
16. Sakthiswary R, Zakaria Z, Das S. Diabetes Mellitus: Treatment challenges and the role of some herbal therapies. Middle-East Journal of Scientific Research. 2014;20(7):786-798.
17. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of Ayurveda. Pharmacognosy reviews. 2014 Jul.;8(16):73.
18. Kavishankar GB, Lakshmidevi N, Murthy SM, Prakash HS, Niranjana SR. Diabetes and medicinal plants-A review. Int. Journal Pharm. Biomed. Sci. 2011;2(3):65-80.

19. Patel DK, Prasad SK, Kumar R, Hemalatha S. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pacific journal of tropical biomedicine. 2012 Apr. 1;2(4):320-330.
20. Chawla R, Thakur P, Chowdhry A, Jaiswal S, Sharma A, Goel R, Sharma J, Priyadarshi SS, Kumar V, Sharma RK, Arora R. Evidence based herbal drug standardization approach in coping with challenges of holistic management of diabetes: a dreadful lifestyle disorder of 21st century. Journal of Diabetes & Metabolic Disorders. 2013 Jul. 4;12(1):35.
21. Mir MS, Darzi MM, Khan HM, Kamil SA, Sofi AH, Wani SA. Pathomorphological effects of Alloxan induced acute hypoglycaemia in rabbits. Alexandria Journal of Medicine. 2013 Dec. 31;49(4):343-353.
22. Lehto S, Ronnemaa T, Haffher SM, Pyorala K, Kallio V, Laakso M. Dyslipidemia and hyperglycemia predict coronary heart disease events in middle-aged patients with NIDDM. Diabetes. 1997 Aug. 1;46(8):1354-1359.
23. Purnell JQ, Hokanson JE, Marcovina SM, Steffes MW, Cleary PA, Brunzell JD. Effect of excessive weight gain with intensive therapy of type 1 diabetes on lipid levels and blood pressure: results from the DCCT. Journal of the American Medical Association 1998 Jul. 8;280(2):140-146.
24. Thorn LM, Forsblom C, Fagerudd J, Thomas MC, Pettersson-Fernholm K, Saraheimo M, Waden J, Ronnback M, Rosengard-Barlund M, AfBjorkesten CG, Taskinen MR. Metabolic syndrome in type 1 diabetes. Diabetes care. 2005 Aug. 1;28(8):2019-2024.
25. Meyer C, Dostou J, Nadkarni V, Gerich J. Effects of physiological hyperinsulinemia on systemic, renal, and hepatic substrate metabolism. American Journal of Physiology-Renal Physiology. 1998 Dec. 1;275(6): F915-921.
26. Bakris G, Vassalotti J, Ritz E, Wanner C, Stergiou G, Molitch M, Nesto R, Kaysen GA, Sowers JR. National Kidney Foundation consensus conference on cardiovascular and kidney diseases and diabetes risk: an integrated therapeutic approach to reduce events. Kidney international. 2010 Oct. 2;78(8):726-736.
27. Eleazu CO, Iroaganachi M, Okafor PN, Ijeh II, Eleazu KC. Ameliorative potentials of ginger (Z. Officinale Roscoe) on relative organ weights in streptozotocin induced diabetic rats. International journal of biomedical science: IJBS. 2013 Jun.;9(2):82.

28. Grover JK, Yadav S, Vats V. Medicinal plants of India with anti-diabetic potential. Journal of ethnopharmacology. 2002 Jun. 30;81(1):81-100.
29. Shrirao A.V. et al Hypolipidemic activity of Madhuca longifolia in triton induced hyperlipidemic rats / International Journal of Biomedical and Advance Research 2017; 8(07): 270-274.
30. Navghare VV, Dhawale SC. In vitro antioxidant, hypoglycemic and oral glucose tolerance test of banana peels. Alexandria Journal of Medicine. 2016 Jul. 25.
31. Navghare V, Dhawale S. Suppression of Type-II Diabetes with Dyslipidemia and Nephropathy by Peels of Musa Cavendish Fruit. Indian Journal of Clinical Biochemistry. 2016 Oct. 1;31(4):380-389.
32. Ghosh R, Dhande I, Kakade VM, Vohra RR, Kadam VJ. Mehra, Antihyperglycemic activity of Madhuca longifolia in alloxan-induced diabetic rats. The Internet Journal of Pharmacology. 2009;6(2):1-2.
33. Dahake AP, Chakma CS, Chakma RC, Bagherwal P. Antihyperglycemic activity of methanolic extract of Madhuca longifolia bark. Diabetologiacroatica. 2010 Mar. 1;39(1):3-8.
34. Prashanth S, Kumar AA, Madhu B, Kumar YP, Prashanth S. Antihyperglycemic and antioxidant activity of ethanolic extract of Madhuca longifolia bark. International Journal of Pharmaceutical Sciences Review and Research. 2010;5(3).
35. Awasthi YC, Mitra CR. Madhuca latifolia.: Constituents of fruit pulp and nut-shell. Phytochemistry. 1967 Jan. 1;6(1):121-125.
36. Singh R, Kaur N, Kishore L, Gupta GK. Management of diabetic complications: a chemical constituents based approach. Journal of ethnopharmacology. 2013 Oct. 28;150(1):51-70.
37. Singh AB, Yadav DK, Maurya R, Srivastava AK. Antihyperglycemic activity of α-amyrin acetate in rats and db/db mice. Natural product research. 2009 Jun. 15;23(9):876-882.
38. Ng TB, Wong CM, Li WW, Yeung HW. Insulin-like molecules in Momordicacharantia seeds. Journal of ethnopharmacology. 1986 Jan. 1;15(1):107-117.
39. Mishra SB, Rao CV, Ojha SK, Vijayakumar M, Verma A. An analytical review of

plants for antidiabetic activity with their phytoconstituent and mechanism of action. Int. Journal Pharm. Sci. Res. 2010;1(1):1647-1652.

40. Das S, Das S, De B. In vitro inhibition of key enzymes related to diabetes by the aqueous extracts of some fruits of West Bengal, India. Current Nutrition & Food Science. 2012 Feb. 1;8(1):19-24.
41. Wang H, Liu T, Song L, Huang D. Profiles and α-amylase inhibition activity of Proanthocyanidins in unripe Manilkara zapota (L). Journal of agricultural and food chemistry. 2012 Mar. 19;60(12):3098-3104.
42. Fayek NM, Monem AR, Mossa MY, Meselhy MR. New triterpenoid acyl derivatives and biological study of Manilkara zapota (L.) Van Royen fruits. Pharmacognosy research. 2013 Apr;5(2):55.
43. Paul SR, Hakim M. In vivo hypoglycemic study of Manilkara zapota leaf and seed extracts. Bangladesh Journal of Pharmacology. 2015 Jan. 1;10(1).
44. Shui G, Wong SP, Leong LP. Characterization of antioxidants and change of antioxidant levels during storage of Manilkara zapota L. Journal of agricultural and food chemistry. 2004 Dec. 29;52(26):7834-7841.
45. Woo PF, Yim HS, Khoo HE, Sia CM, Ang YK. Effects of extraction conditions on antioxidant properties of sapodilla fruit (Manilkara zapota). International Food Research Journal. 2013;20(5):2065-2072.
46. Xiao JB, Hogger P. Dietary polyphenols and type 2 diabetes: current insights and future perspectives. Current medicinal chemistry. 2015 Jan. 1;22(1):23-38.
47. Shirosaki M, Koyama T, Yazawa K. Anti-hyperglycemic activity of kiwifruit leaf (Actinidia deliciosa) in mice. Bioscience, biotechnology, and biochemistry. 2008 Apr. 23;72(4):1099-1102.
48. Yang H, Lee YC, Han KS, Singh H, Yoon M, Park JH, Cho CW, Cho S. Green and gold kiwifruit peel ethanol extracts potentiate pentobarbital-induced sleep in mice via a GABAergic mechanism. Food chemistry. 2013 Jan. 1;136(1):160-163.
49. Lim TK. Actinidia deliciosa. Edible Medicinal and Non-Medicinal Plants. Springer Netherlands. 2012; 1:20-29.
50. Soren G, Sarita M, Prathyusha T. Antidiabetic activity of Actinidia deliciosa fruit in alloxan induced diabetic rats. The Pharma Innovation. 2016 Sep. 1;5(9, Part A):31.

51. Fiorentino A, D'Abrosca B, Pacifico S, Mastellone C, Scognamiglio M, Monaco P. Identification and assessment of antioxidant capacity of phytochemicals from kiwi fruits. Journal of agricultural and food chemistry. 2009 Apr. 9;57(10):4148-4155.
52. Lee DE, Shin BJ, Hur HJ, Kim JH, Kim J, Kang NJ, Kim DO, Lee CY, Lee KW, Lee HJ. Quercetin, the active phenolic component in kiwifruit, prevents hydrogen peroxide-induced inhibition of gap-junction intercellular communication. British journal of nutrition. 2010 Jul.;104(2):164-170.
53. Yang H, Lee YC, Han KS, Singh H, Yoon M, Park JH, Cho CW, Cho S. Green and gold kiwifruit peel ethanol extracts potentiate pentobarbital-induced sleep in mice via a GABAergic mechanism. Food chemistry. 2013 Jan. 1;136(1):160-163.
54. Chang HY, Wallis M, Tiralongo E. Use of complementary and alternative medicine among people living with diabetes: literature review. Journal of advanced nursing. 2007 May 1;58(4):307-319.
55. Manya K, Champion B, Dunning T. The use of complementary and alternative medicine among people living with diabetes in Sydney. BMC complementary and alternative medicine. 2012 Jan 12;12(1):2.
56. Ghorbani A. Clinical and experimental studies on polyherbal formulations for diabetes: current status and future prospective. Journal of integrative medicine. 2014 Jul. 1;12(4):336-345.
57. Bader, ghulam. Current status of herbal drugs used in the treatment of diabetes mellitus. World journal of pharmaceutical research.2017; 6: 323-341.
58. Ghadge AA, Kuvalekar AA. Controversy of oral hypoglycemic agents in type 2 diabetes mellitus: Novel move towards combination therapies. Diabetes & Metabolic Syndrome: Clinical Research & Reviews. 2016 Aug. 22.
59. Hajianfar H, Paknahad Z, Bahonar A. The effect of omega-3 supplements on antioxidant capacity in patients with type 2 diabetes. Int. Journal of Preventive Medicine. 2013;4 (2):234–238.
60. Dey L, Attele AS, Yuan CS. Alternative therapies for type 2 diabetes. Alternative Med. Rev. 2002; 7: 45-58.
61. Gupta PD, De A. Diabetes Mellitus and its herbal treatment. International Journal of Research in Pharmaceutical and Biomedical Sciences. 2012; 3(2): 706-721.

62. Mamun-or-Rashid A, Hossain MS, Naim Hassan B, Kumar Dash M, Sapon A, Sen MK. A review on medicinal plants with antidiabetic activity. Journal of Pharmacognosy and Phytochemistry. 2014; 3(4):149-159.
63. Hegazy GA, Alnoury AM, Gad HG. The role of Acacia Arabica extract as an antidiabetic, antihyperlipidemic, and antioxidant in streptozotocin-induced diabetic rats. Saudi medical journal. 2013; 34(7):727-733.

DR. DHAWALE S.C.

Ph.D. Supervisor

MR. RATHOD S.G.

Research Student